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Product Information

High Sensitivity C-Reactive Protein (CRP) ELISA

Catalog Number **SE120041**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

C-Reactive protein (CRP) is an alpha globulin with a molecular mass of 110,000–140,000 Daltons and is composed of five identical subunits, which are noncovalently assembled as a cyclic pentamer. CRP is synthesized in the liver and is normally present as a trace constituent of serum or plasma at levels less than 0.3 mg/dl. C-Reactive protein is one of the acute-phase proteins, the serum or plasma levels of which rise during general, nonspecific response to a wide variety of diseases. Although the detection of elevated levels of CRP in the serum is not specific for any particular disease, it is a useful indicator of inflammatory processes. Additionally, measurement of CRP by high-sensitivity C-Reactive protein assays may add to the predictive value of other cardiac markers (myoglobin, creatine-kinase-MB, troponin I and T), which are used to assess the risk of cardiovascular and peripheral vascular disease. Inflammation in the arteries may play a role in heart disease and HS-CRP can determine heart disease risk in those with undetected heart disease and risk of complications for those who have already had a heart event.

The High Sensitivity C-Reactive Protein ELISA Kit is intended for the quantitative determination of C-reactive protein (CRP) in human serum or plasma.

The CRP ELISA kit is a solid phase direct sandwich method. The samples and anti-CRP-HRP conjugate are added to the wells coated with MAb to CRP. CRP in the patient's serum binds to anti-CRP MAb on the well and the anti-CRP second antibody then binds to CRP. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of CRP in the samples. A standard curve is prepared relating color intensity to the concentration of the CRP.

Components

Materials Provided	96 Tests
Microwells coated with CRP MAb	12 × 8 × 1
CRP Standard: 6 vials (ready to use)	0.25 ml
CRP Enzyme Conjugate: 1 bottle (ready to use)	12 ml
TMB Substrate: 1 bottle (ready to use)	12 ml
Stop Solution: 1 bottle (ready to use)	12 ml
Sample Diluent	50 ml
20× Wash concentrate: 1 bottle	25 ml

Reagents and Equipment Required but Not Provided.

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450 nm
- Absorbent paper or paper towel
- Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at 2–8 °C for 5 days. If storage time exceeds 5 days, store frozen at –20 °C for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

20× Wash Buffer Concentrate
Prepare 1× Wash buffer by adding the contents of the bottle (25 ml, 20×) to 475 ml of distilled or deionized water. Store at room temperature (18–26 °C).

Storage/Stability
Store the kit at 2–8 °C.

Procedure
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that standards, control and serum samples be run in duplicate.

Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Prior to assay, allow reagents to stand at room temperature (18–26 °C).

1. Gently mix all reagents before use.
2. Place the desired number of coated strips into the holder
3. Dilute patient samples and controls 100-fold by adding 5 µl of sample or control to 495 µl of Sample Diluent
   Note: Standards are ready to use, do NOT dilute.
4. Dispense 10 µl of standard, diluted samples and controls into the appropriate wells
5. Add 100 µl of enzyme conjugate to all wells. Tap the holder to remove air bubbles from the liquid and mix well.
6. Incubate for 60 minutes at room temperature (18–26 °C).
7. Remove liquid from all wells. Wash wells 3 times with 300 µl of 1× wash buffer. Blot on absorbent paper towels.
8. Add 100 µl of TMB Substrate to all wells.
9. Incubate for 15 minutes at room temperature.
10. Add 50 µl of Stop Solution to all wells. Shake the plate gently to mix the solution.
11. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the Stop Solution.

Results
Calculations
The standard curve is constructed as follows:
1. Check CRP standard value on each standard vial. This value might vary from lot to lot. Make sure the value is checked on every kit.
2. To construct the standard curve, plot the absorbance for the CRP standards (vertical axis) versus the CRP standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.

   Example of a Standard Curve

	OD 450 nm	Concentration mg/L
Std 1	0.02	0
Std 2	0.23	0.005
Std 3	0.49	0.01
Std 4	1.01	0.025
Std 5	1.66	0.05
Std 6	2.40	0.1

3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.
4. The obtained values of the patient samples and control sera should be multiplied by the dilution factor of 100 to obtain CRP results in mg/l.
   Notes: Patient samples with CRP concentrations greater than 10 mg/l should be further diluted 10-fold after the initial 100-fold dilution (total dilution 1,000-fold), and the final CRP values should be multiplied by 1,000 to obtain CRP results in mg/L.

   The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings, and other diagnostic procedures.

Expected Values
It is recommended that each laboratory establish its own normal range based on the patient population. However, based on published literature healthy individuals are expected to have CRP values as follows: the CRP level in normal human serum ranges from 0.2–10 mg/L, where 90% of apparently healthy individuals have CRP levels <3 mg/L and only 1% have levels >10 mg/L.

Product Profile

Correlation with a Reference ELISA kit:
A total of 84 sera were tested by this ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.93	0.72	0.011

Precision
Intra-Assay

Serum	Number of Replicates	Mean (mg/L)	Standard Deviation	Coefficient of Variation (%)
1	16	0.004	0.0002	5.06
2	16	0.021	0.0011	5.28
3	16	0.008	0.0008	9.59

Inter-assay

Serum	Number of Replicates	Mean (mg/L)	Standard Deviation	Coefficient of Variation (%)
1	10	0.004	0.0003	8.51
2	10	0.009	0.0007	8.34
3	10	0.020	0.0016	7.95

Recovery
Known quantities of CRP were added to a serum that contained a low concentration of CRP.

Expected Value (mg/L)	Recovered (mg/L)	Percentage of Recovery
0.005	0.0053	106
0.0125	0.0102	82
0.025	0.0236	94

Linearity
Two different patient samples were diluted with the "0" calibrator to 1:2, 1:4 and 1:8. CRP values were assayed and results were corrected with the dilution factor. The results of these dilution tests are as follows:

Serum	Original Value (mg/L)	Percentage of Recovery		
		1:2	1:4	1:8
1	0.032	94	100	100
2	0.041	93	88	117
3	0.095	84	84	82

References

1. Schultz, D.R., and Arnold, P.I., Properties of four acute phase proteins: C-reactive protein, serum amyloid A protein, glycoprotein, and fibrinogen. Seminars in Arthritis and Rheumatism, **20**, 129-147 (1990).
2. Kindmark, C.O., The Concentration of C-reactive protein in Sera from Healthy Individuals. Scand. J. Clin. Lab. Invest., **29**, 407- 411 (1972).
3. Dowling, P., and Cook, S., Immune events in demyelinating disease. in Multiple sclerosis, Wolfgang, F. et al., (eds.), Academic Press Inc., (New York, NY: 1972) 269-277.
4. Yudkin, J.S. et. al., C-Reactive Protein in Healthy Subjects: Association with Obesity, Insulin Resistance, and endothelial dysfunction. A potential role for cytokines originating from adipose tissue? Arterioscler. Thromb. Vasc. Biol., **19**, 972-8 (1999).
5. Kushner, I., and Rzewnicki, D.L., The acute phase response: General aspects. Bailliere's Clinical Rheumatology, **8**, 513-530 (1994).
6. Macy, E.M. et al., Variability in the measurement of C-reactive protein in healthy subjects: implications for reference interval and epidemiological applications. Clin. Chem., **43**(1), 52-58 (1997).
7. Hedlund, P., Clinical and experimental studies on C-reactive protein (acute phase protein). Thesis Acta Med. Scand., **128**(Suppl. 361), 1-71 (1961).
8. Hedlund, P., The appearance of acute phase protein in various diseases. Acta Med. Scand., **128**(Suppl. 196), 579-601 (1947).
9. Morley, J.J., and Kushner, I., Serum C-reactive Protein Levels. in C-Reactive Protein and the Plasma Protein Response to Tissue Injury, Kushner, I. et al., eds., Annals of N.Y. Acad. Sci., **389**, 406-417 (1982).

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